Date of Initial Use _____
Date Retired _____
(Retain for 2 years)

Procedure Manual

Test Name: Wet Mounts/KOH Preps

Method or Principle:
Performing a microscopic examination for the presence of Trichomonas vaginalis, clue cells, and yeast in a saline suspension of vaginal secretions. Vaginitis is one of the most commonly encountered complaints of the female patient. The majority of cases, approximately 90% are caused by Candida, Gardnerella, or Trichomonas. Diagnosis and effective treatment depend upon accurate identification of the etiologic agent, effective specific therapy, and restoration of the normal ecosystem of the vagina.

Trichomonas vaginalis is a parasite that is distinguished by its rapid movement and flagella. Motile Trichomonas trophozoites may be identified in a vaginal sample by its characteristic structure.

Bacterial vaginosis is the most common type of vaginal infection and can sometimes be detected by the presence of "clue cells." Clue cells are epithelial cells entirely covered with bacteria giving the cell a "fur-like" appearance. If the organisms are sticking to the edges or on top of the cell, without extending past the cytoplasmic margins, a diagnosis of clue cells cannot be made. Note: Certain anaerobic, non-pathologic, species tend to adhere to the epithelial surface.

Yeast infections are primarily caused by Candida albicans, although other Candida species are becoming increasingly important as disease agents. Candida albicans, in low numbers, is considered part of the normal vaginal flora, but may proliferate to cause an infection. Yeast should be observed with the addition of Potassium Hydroxide (KOH) for the presence of budding or pseudohyphae forms.

Specimen Collection:

a) Requirements for patient preparation: There is no patient preparation needed. The patient should be warned not to douche for 24 hours before the test.
b) Specimen type: vaginal swab immersed in vial containing 2 mL sterile 0.9% Sodium Chloride (NaCl)
c) Labeling requirements: 2 unique identifiers on primary and all secondary containers (name, DOB, or EMR, SS, phone#)
d) Storage conditions and limits: room temperature within 20 minutes
e) Transportation instructions: room temp, within 20 minutes
f) Process promptly for the presence of Trichomonas vaginalis, clue cells, and yeast.
g) Referral to outside lab – not possible
h) Criteria for rejection – any specimen not tested within 20 minutes

Preparation and Storage:

a) 10% KOH, shelf life is one year. Store at room temperature.
b) 0.9% Sterile NaCl, expiration date is one year from the date vials are prepared. Store at room temperature.

Microscopic examination:

a) Trichomonas
   Scan the entire slide on low power magnification with reduced light for motile Trichomonas. If motility is observed, switch to high power to positively identify. The flagella or undulating membrane should be visible. If seen, report as present. If motile Trichomonas are not seen, report as negative.
b) Clue cells
   Clue cells are epithelial cells of the vagina covered with bacteria giving the cell a "fur-like" appearance. Clue cells are reported as: Few, Moderate, or Many on Low power.
c) Yeast
   The wet prep with KOH should be used to determine if yeast are present. Budding yeast and pseudohyphae, if observed, are estimated on high power. Record as Few, Moderate, or Many on High Power Field. If no yeast is seen, report as no yeast observed.

Calibration: Not Applicable

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Control procedures

a) Check the reagent expiration date prior to use.
b) Quality control for wet prep and KOH microscopic examinations may take the form of an elemental comparison with a chart or reference book pictures illustrating white blood cells, red blood cells, epithelial cells, bacteria, Trichomonas, clue cells, budding yeast or hyphae.

Test performance

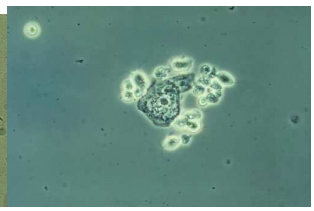
a) **Wet prep**: Mix sample in vial and place one drop of the solution onto a clean glass slide, cover with coverslip and examine microscopically using high power (40x) objective for the presence of Trichomonas and clue cells.
b) **Wet prep with KOH**: Mix sample in vial and place two drops of the solution into a small test tube.
Add two drops of 10% KOH to the tube.
Mix and allow to sit for about 5 minutes until the material has cleared. Additional time may be required for thicker samples.
Place one drop on the slide and examine microscopically for the presence of budding yeast and/or pseudohyphae forms.

c) Interpretation of results

When drops of a 10 percent KOH solution are added to the vaginal secretions of a woman with bacterial vaginosis, an amine, or "fishy" odor is released. This test, commonly referred to as a "whiff test," is positive in women with bacterial vaginosis, but can also be positive in patients with Trichomonas infection.
Another diagnostic criterion for bacterial vaginosis is the presence of clue cells on wet mount. Clue cells are vaginal epithelial cells that have a stippled appearance due to adherent coccobacilli. The edges of the cells are obscured and appear fuzzy compared with the normally sharp edges of vaginal epithelial cells. To be significant for bacterial vaginosis, more than 20 percent of the epithelial cells on the wet mount should be clue cells.
The wet mount usually does not show the increased number of leukocytes seen in other types of vaginitis. If the wet mount shows increased numbers of leukocytes, a co-infection (e.g., Trichomonas) should be suspected.
Remaining silent on the presence of Trichomonas, clue cells and yeast is interpreted as not being observed.


Clue Cells


Hyphae


Trichomonas

Reportable range

a) Trichomonas: Negative, Present
b) Clue cells: Negative, Few (1-10), Moderate (10-50), Many (>50) / lpf
c) Yeast: No yeast observed, Few (1-10), Moderate (10-50), Many (>50) Budding, or Pseudohyphae / hpf

Limitations

a) Poor sample collection technique.
b) Delay in testing will cause deterioration of elements.

Reference Range

a) Negative Trichomonas.
b) Candida albicans is considered normal vaginal flora, but may proliferate to cause an infection.
c) Negative for clue cells.

Panic Protocol

a) Unnecessary for physician performed microscopy procedure (PPMP)-certified laboratories as the provider is already notified per definition of PPMP.

References:

a) Henry, J B: Clinical Diagnosis and Management by Laboratory Methods, 19th ed., W.B. Saunders Company, Philadelphia, 1996.
b) Kern, M: Medical Mycology, Philadelphia, F.A. Davis Company, 1990.
c) Loventhal, R.: Medical Parasitology, 3rd ed., Philadelphia, F.A. Davis Company, 1989.

Reporting Results

Results are entered into the patient medical record. The physician makes a clinical correlation for appropriate results.
Corrected report policy and procedure. Add comment "Previously reported as __ on __(date)".
Note: Maintain all preliminary, final, corrected reports for 2 years.

Test System Inoperable

a) Description of the course of action to take if a **test system** becomes inoperable.
    a) Call service representative for microscope repair
    b) Use alternate microscope

b) Description of the course of action to take if a **reporting system (**EMR or LIS) becomes inoperable.

Not applicable or

    a) Hold all results until information system is operational.
    b) Use backup plan of manual entry and reporting system and delivery

Note: All handwritten changes to procedures must be signed and dated by the laboratory director before being implemented.

Approved

_____ Date _____
Signature Laboratory Director